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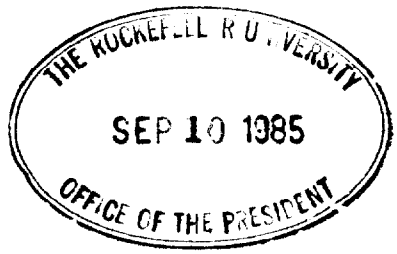


Dr. Joshua Lederberg, President
The Rockefeller University
1230 York Avenue
New York, NY 10021-6399

Dear Dr. Lederberg:

I have appreciated your thoughts on the issue of DNA stability and rearrangement that you communicated to Peter Kretschmer. We wanted to let you know about some of our recent work on the muconic acid production strain of Pseudomonas putida with regard to the DNA stability phenomenon.

As you know, in fermentation runs with the muconic acid production strain, we found that the cells increase production rate to a maximum within 24h, then the cells enter a period of rapid decline in production rate (see Fig. 1). When we tested several isolates from late in the fermentation run for the presence of the TOL genes by Southern hybridizations, we found that they had deleted the region of DNA that encodes the xylABC genes which convert toluene to benzoic acid. This region of DNA is a 39kb region flanked by 1.4kb direct repeats and is known to undergo deletion from the TOL plasmid pWW0 during growth on benzoate of P. putida containing pWW0 (Molec. Gen. Genet. 184:97 (81), see Fig. 2).

Interestingly, we determined that the decline appears to involve a physiological effect initially (at this point >99% of the cells in the fermenter contain the xylABC genes), and approximately 24 hours after the initial decline in activity, there is an equally precipitous drop in the percentage of cells that still contain the xylABC genes (see Fig. 1). In this case the percentage of cells containing the xylABC genes was determined by plate matings in which a donor strain of P. putida mobilizes a plasmid containing cloned catBCDE genes into isolates purified at various stages from the fermentation. The cloned genes complement the catB and pcaE mutations in the production strain and the TOL+ phenotype of clones that still contain the deletable genes (xylABC) can then be scored by testing growth on toluene.

Issues:

1) Why is there this apparent two-stage effect of loss of activity followed by loss of genetic information? Programming, perhaps, as per your suggestions?

Issues: (continued)

2) The rate of loss of xylABC DNA is much too rapid for simple selection of deleted derivative cells by growth rate, since there is little or no cell growth at this stage of the fermentation. Such an effect could indeed be the result of programmed DNA loss.

3) Are the 1.4kb direct repeat regions essential recognition sites for excision, and if so, could excision be precluded by removal of one or both repeats?

4) What is responsible for the physiological decline in activity?

5) Are the physiological and genetic effects related, for example, such that the xylABC genes are excised causing gene expression to turn off, but the excised DNA is still contained within the cell for a period of time and can be recovered by mating?

This last idea seems unlikely to me and our current hypotheses are that there is initially the depletion of an essential nutrient or the accumulation of a toxic substance (muconic acid?) causing inhibition of activity, and that for some unknown reason deletion of the xylABC genes follows shortly thereafter.

We have cloned a 25kb fragment of DNA from pWW0 that contains xylABC (and probably all of one of the direct repeats) in E. coli. (Fig. 3). Interestingly, it is unstable also, and we see the formation of deletion derivative plasmids within as short a period as 24 hours. These clones do not grow well on complex or minimal medium, and I am inclined to think that it is the presence of the enzymes (toluene monooxygenase, benzyl alcohol dehydrogenase, benzaldehyde dehydrogenase) in E. coli that leads to instability here, since cells with intact plasmid are sick, and healthy derivatives were shown to have deleted most or all of the 25kb insert. I am currently working on subcloning a smaller fragment that contains the xylABC genes into a wide host range vector that will permit transfer of the cloned genes into our P. putida production strain.

The examples of bacterial programmed DNA loss are certainly interesting phenomena, and if this programming is involved in TOL DNA, then it will, I think, represent a somewhat different mechanistic subclass of programmed DNA loss, because the effect of this deletion is to remove structural gene DNA rather than remove DNA in order to regulate expression of specific genes. Ordinarily, removal of structural genes would not appear to benefit the cell, unless there is some unknown disadvantage to maintaining these genes under certain growth conditions in nature.

If we are able to prevent deletion of the DNA, then it will be interesting to see what effects, if any this may have on the cells, both from the production aspect and on cell physiology.

Once again, thank you for your input, and I trust this letter brings you up to date on our experiences with this interesting phenomenon.

Sincerely,

Randall A. Roehl, Ph. D.
Senior Research Geneticist

RAR:mc
attachment

cc: P. Kretschmer
P. Maxwell

Fig. 1

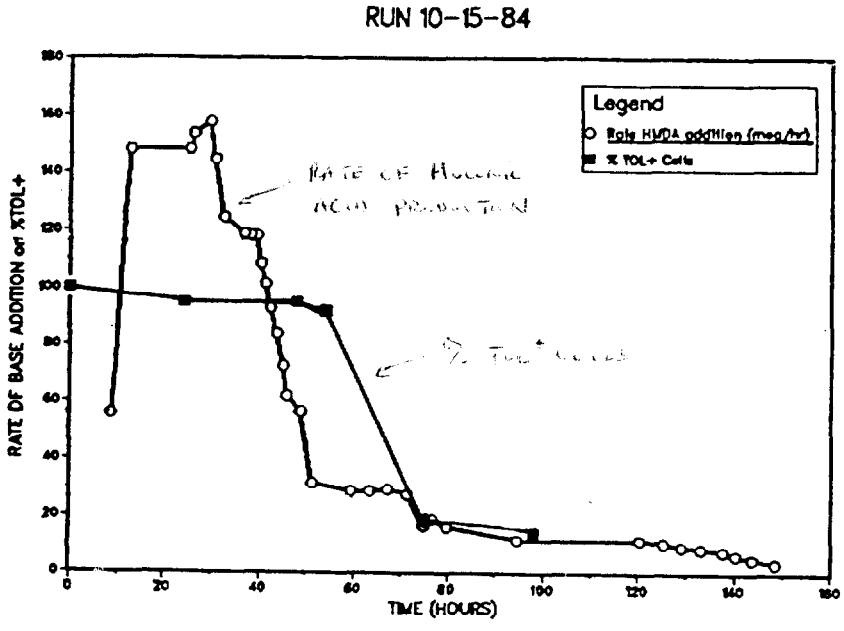


Fig. 2

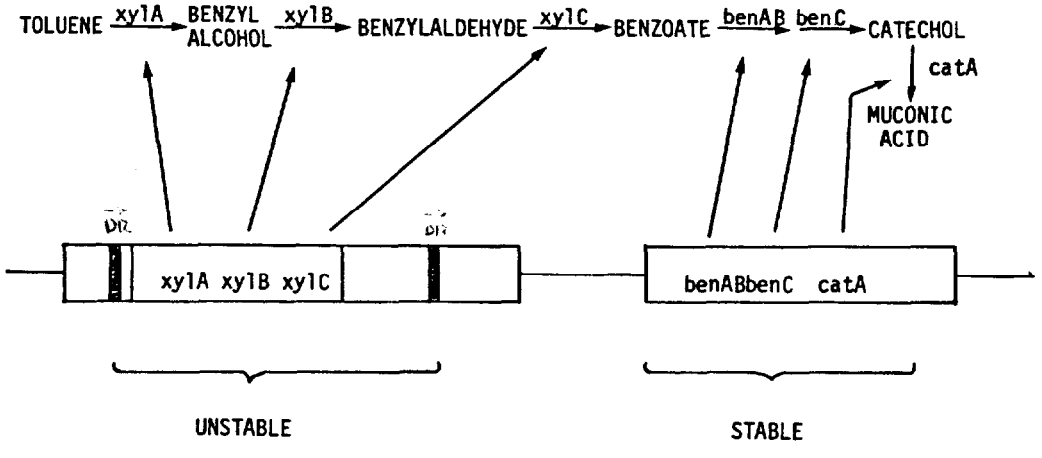


Fig. 3